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Glutathione Transferase Theta 1-1-Dependent Metabolism of the Water Disinfection Byproduct Bromodichloromethane

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INTRODUCTION

The trihalomethanes are disinfection by-products (DBPs) found in drinking water following chlorination procedures; as a class, the trihalomethanes are the most abundant DBPs detected. Bromodichloromethane (BDCM or $CHBrCl_2$) is second only to chloroform ($CHCl_3$) in prevalence among DBPs in U.S. chlorinated drinking water and is a more potent carcinogen and toxicant than $CHCl_3$. The carcinogenic potency of BDCM, its genotoxicity, and its greater propensity to be metabolically activated are all factors which have made BDCM one of the highest priority DBPs for research. By analogy to the dihalomethanes and dihaloethanes, the GST-mediated metabolic pathway for trihalomethanes is expected to contribute only a minor fraction quantitatively to the overall metabolism of these compounds. However, toxic reactive intermediates may be generated with the potential to modify cellular nucleophiles.

METHODS

Radiometric assay (Fig.1,2). Biotransformation kinetics of [^{14}C]$CHBrCl_2$ by cytosols and pure recombinant rat GST T1-1 were measured by a radiometric assay. Reaction mixtures contained rodent, human, or *S. typhimurium* RSJ100 cytosol (1-8 mg protein/mL) supplemented with 10 mM GSH and various amounts of [^{14}C]$CHBrCl_2$ (0–30 mM). Cytosol concentrations of 4 mg cytosol protein/mL and incubation times of 60 min (at 37°C) were routinely used as a result of time- and protein-dependence experiments. Pure recombinant rat GST T1-1 was used instead of cytosols in select experiments. Reactions were terminated by adding 2% Na_2CO_3/1% NaCl (w/v) and the aqueous solutions were extracted with ethyl acetate (3 x 0.4 mL) to remove unreacted [^{14}C]$CHBrCl_2$. The remaining aqueous fraction was radioassayed to measure rates of formation of GSH-derived products.

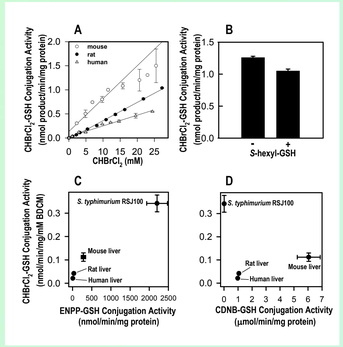
HPLC analysis of recombinant rat GST T1-1-catalyzed reaction products (Fig.3). The aqueous ^{14}C-labeled products (25 μL) were adjusted to pH<2 and authentic *S*-formyl-GSH was added. The mixture was injected onto a reversed-phase HPLC column and the column developed with a H_2O/ACN linear gradient containing 0.06% TFA. Column eluent was monitored in series by a UV detector (235 nm) and a radioactivity flow detector.

Recombinant rat GST T1-1 incubations supplemented with NAD$^+$ (Fig.4). Incubations of [^{14}C]$CHBrCl_2$ with recombinant rat GST T1-1 were supplemented with NAD$^+$ (1 mM) and GSH-dependent formaldehyde dehydrogenase. Control reactions contained all components except NAD$^+$. The reactions were processed and analyzed for [^{14}C]HCO_2H as described in Fig.4. (The overall recovery of formate by this procedure was estimated to be 69.1 ± 6.1%).

HPLC/MS analysis (Fig.5,6). *In vitro* reaction products were analyzed with an ion-trap mass spectrometer to aid identification of $GSCH_2OH$. The GSH conjugation reaction conditions were similar to those described above.

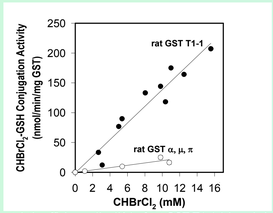
RESULTS

Fig. 1. Rodent and human liver cytosolic catalysis of GSH conjugation of $CHBrCl_2$ and the major role of GST T1-1.



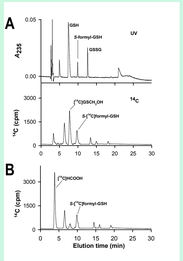
(A) $CHBrCl_2$-GSH conjugation activity of mouse, rat, and human cytosols determined by the radiometric assay. Rank order was mouse>rat>human. (B) Inhibition of aggregate GST alpha, mu, and pi activity in mouse liver cytosol by *S*-hexyl-GSH during biotransformation of 10 mM [^{14}C]$CHBrCl_2$. *S*-Hexyl-GSH had only a modest effect on $CHBrCl_2$-GSH conjugation activity. (C) Correlation of GST T1-1 activity (ENPP-GSH conjugation) with $CHBrCl_2$-GSH conjugation activity. (D) Lack of correlation of GST alpha, mu, and pi activity (CDNB-GSH conjugation) with $CHBrCl_2$-GSH conjugation activity. (Each symbol, *n*=2-3).

Fig. 2. $CHBrCl_2$-GSH conjugation reactions catalyzed by purified GST T1-1 or GSTs alpha, mu, and pi.



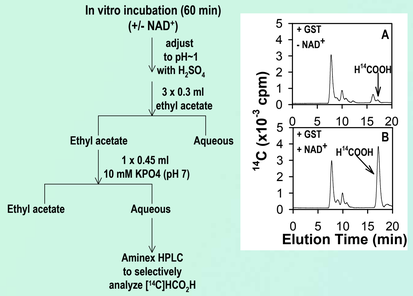
The catalytic efficiency (k_{cat}/K_m) of rat GST T1-1 (closed circles) was 0.4 mM^{-1} min^{-1}. By comparison, k_{cat}/K_m for CH_2Cl_2 is ~3–6-fold greater reflecting its greater reactivity with GSH. The catalytic efficiency of rat GSTs alpha, mu, pi (open circles) was 0.06 mM^{-1} min^{-1}. (Each symbol, *n*=2).

Fig. 3. ^{14}C-Labeled metabolites following GSH conjugation of [^{14}C]$CHBrCl_2$ analyzed by HPLC: Identification of *S*-formyl-GSH, $GSCH_2OH$, HCO_2H.



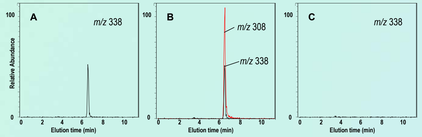
(A) Products resulting from GSH conjugation of [^{14}C]$CHBrCl_2$ in the presence of recombinant rat GST T1-1 were co-chromatographed with authentic *S*-formyl-GSH and examined by HPLC/UV (*top*) and HPLC/RAD (*bottom*). (B) HPLC/RAD analysis of ^{14}C-labeled metabolites (shown in A) after treatment with GSH-dependent formaldehyde dehydrogenase. The peak designated as [^{14}C]$GSCH_2OH$ in (A) was selectively converted to [^{14}C]HCOOH suggesting the following reaction: $GSCH_2OH \rightarrow GSC(=O)H \rightarrow HCO_2H$.

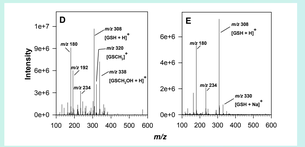
Fig. 4. Supplementation of *in vitro* $CHBrCl_2$-GSH reactions with NAD$^+$ increases [^{14}C]HCOOH formation.



HPLC radiochromatograms of final aqueous extracts of ethyl acetate fractions (see flow diagram, left) following recombinant rat GST T1-1-catalyzed GSH conjugation of [^{14}C]$CHBrCl_2$ in the absence (A) or presence (B) of NAD$^+$ (1 mM). Both reactions were supplemented with GSH-dependent formaldehyde dehydrogenase. [^{14}C]HCOOH peak identification was confirmed by co-elution with authentic HCOOH (measured at 210 nm).

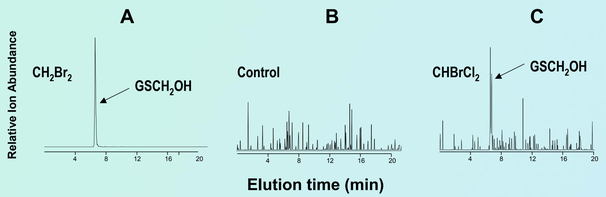
Fig. 5. HPLC-MS of equimolar mixture of GSH and HCHO: *S*-(hydroxymethyl)GSH ($GSCH_2OH$) co-elutes with unmodified GSH by HPLC.





(A-C) LC-MS (m/z 338) analysis of $GSCH_2OH$. (A,B) Analysis of an equimolar mixture of HCHO and GSH following equilibration. Unmodified GSH and $GSCH_2OH$ co-chromatograph with each other as shown in panel B. (C) GSH only; no m/z 338 was detectable. (D,E) Full-scan mass spectra of $GSCH_2OH$ (D) and unmodified GSH (E).

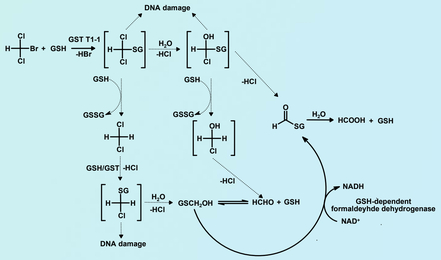
Fig. 6. HPLC-MS/MS (m/z 338→m/z 308) of *in vitro* reactions: Detection of *S*-(hydroxymethyl)GSH following GSH conjugation of CH_2Br_2 or $CHBrCl_2$ catalyzed by pure GST T1-1.



(A) Positive control: CH_2Br_2 + GSH + GST. (B) Negative control: GSH and GST only. (C) Reaction: $CHBrCl_2$ + GSH + GST. SRM m/z 338→m/z 308 transition was continuously monitored throughout the chromatographic run. $GSCH_2OH$ was detectable in the $CHBrCl_2$ reaction.

Scheme 1. Proposed GST T1-1-catalyzed GSH conjugation of $CHBrCl_2$.

Bold arrows indicate pathways that are supported by direct experimental evidence. Dashed arrows indicate pathways that are inferred from the experimental evidence and from literature precedents.



CONCLUSIONS

1. GST T1-1 is the primary catalyst responsible for GSH conjugation of the disinfection byproduct $CHBrCl_2$. Alpha, mu, and pi are poor catalysts.

2. The species rank order of hepatic conjugation of $CHBrCl_2$ is mouse>rat>human. This reflects the the relative GST T1-1 expression levels amongst these species.

3. *S*-formyl-GSH, *S*-(hydroxymethyl)GSH, and HCO_2H have been identified as GST-derived products of $CHBrCl_2$. Several potential reactive intermediates are proposed in Scheme 1 to account for the bacterial mutagenicity of $CHBrCl_2$.

4. Comparison to dichloromethane, which reacts ~10x faster with GSH than $CHBrCl_2$ yet has a similar mutagenic potency in *Salmonella* as $CHBrCl_2$, suggests that the reactive intermediates derived from GSH conjugation of $CHBrCl_2$ (which includes a unique dichloromethyl GSH intermediate) are more reactive.

IMPACT

The kinetic parameters of GSH conjugation of $CHBrCl_2$ and the estimated level of DNA adducts that result has enabled the extension of an existing rodent PBPK model. Thus evaluation of the human cancer risk assessment of $CHBrCl_2$ can be improved by estimating metabolic rates of bioactivation pathways and the levels of DNA adducts produced in relevant tissues following environmental exposures.

FUTURE DIRECTIONS

Other brominated trihalomethanes found in drinking water (e.g., $CHBr_2Cl$, $CHBr_3$) can also be bioactivated by GSH conjugation and produce mutations in *Salmonella*. Their rates of GSH conjugation and their ability to interact with DNA will be investigated. These data can be compared to the mutagenic potencies for each compound and enable correlations to be drawn. Alternative *in vitro* approaches to study the metabolism of these compounds, e.g. the use of rat and human hepatocytes, will also be explored.